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Influence of Sustainable Biocoagulants *Trigonella Foenum – graecum* and *Moringa Oleifera* for Improving Water Potability

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ABSTRACT

Clarification of Drinking water requires complex and costly treatment procedures. Alternative cheap and sustainable methods for water treatment are desired for developing nations. Seed extracts of *Trigonella foenum graecum* and *Moringa oleifera* possess biocoagulant proteins to make water free from physicochemical and microbiological components following Coagulation-flocculation process. In order to verify their capacity for clarity, the raw and defatted seed coagulants were put through the Jar test equipment. The results demonstrated that the dissolved organic carbon in raw water could be more effectively removed using the defatted coagulants (ethanol extracted). Simultaneously the seed coagulant proteins of *M. oleifera* and *Trigonella* had potent bactericidal action, effective in reducing bacterial load by 96% and 84% from surface water respectively. Significant impact on turbidity, pH, TS EC, DO and BOD were observed which focuses on good performance in water treatment. These results indicate usage of these biocoagulants alone or in conjunction with chemical coagulants as coagulant aid, which can substantially improve water potability and hence reduce prevalence of water borne diseases.

KEYWORDS: *Trigonella, Moringa Coagulation, Flocculation, Turbidity*

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1. INTRODUCTION

Potable water availability and accessibility is a global challenge to developing and underdeveloped nations which are facing hard core water crisis. According to United Nations (UN) World Water Development Report (WWDR, 2018), this pandemonium will escalate further by the end of 2050. World Health Organization (WHO) also highlights that 80% global disease burden originates from water related sources involving failure or slack in hygiene and Wash techniques. Moreover, excessive demand placed on finite water sources compounded with human inflicted pollution, as well as inadequate and inefficient measures to handle and dispose aquatic waste has worsened the situation. Usually, physical, chemical and microbiological standards are used to evaluate water potability for human consumption.

Surface water treatment procedures employ Flocculation-coagulation mechanism which works by reducing turbidity and destabilize dissolved and suspended materials from water into large aggregates which separates from water by settling down. Coagulation is a chemical process which involves charge neutralization whereas flocculation is solely a physical process. The processes of turbidity removal eliminate suspended particles and attached microbial load minimizing the risk of waterborne infections like amoebic dysentery, giardiasis, cholera, jaundice and typhoid. Chemical coagulants works wonder in small dosages on the coagulation-flocculation principle and leads to 99% removal of turbidity and associated pollutants, but has detrimental effect on human and ecosystem. Chemical coagulant generates voluminous sediment sludge after treatment which imposes health hazard whereas natural coagulants positively affect the ecosystem and do not change the pH of sample water compared to chemical coagulants (Jayalakshmi *et al.*, 2017; Deshmukh *et al.*, 2018; Yusoff *et al.*, 2019; Kurniawan *et al.*, 2020). In household scale,

chemical coagulants like alum is widely preferred but long and repeated use results in Alzheimer's disease (Gauthier *et al*., 2000). Thus there is an utter need to identify and develop sustainable green systems to generate safe drinking water by which rural communities can be benefitted at large.

Turbidity removal involving coagulation process is augmented by several controlled parameters like initial turbidity, contact time, pH, rotation speed, biocoagulant dose and its nature (Kazi and Virupakshi, 2013; Choubey et al., 2012; Hashim and Al-Mufti, 2013; Issa and Babiker, 2014; Megersa *et al*, 2016). Plant based seeds and fruits acts as noble coagulant-flocculants for treating various effluent wastes like tannery, palm oil, abattoir, paper and pulp (Shabanizadeh and Taghavijeloudar, 2023), landfill leachate (Zainal *et al*., 2021). Some researchers have also proposed blend of natural and chemical flocculants like alum to increase the water turbidity removal efficiency (Subramonian *et al*., 2015; Shak and Wu, 2015; Li *et al*., 2020). Certain biocoagulants not only harbour coagulation capabilities, but due to phytochemicals like phenols and flavonoids exhibit bactericidal properties also (Choy *et al*., 2014). Hence in addition to turbidity removal, pathogenic strains of *Escherichia coli* found in surface water are also eliminated by the coagulating settling sludge.

Trigonella foenum graecum, commonly, Fenugreek belongs to the Fabaceae family, ubiquitous of Asia, Middle East and the Northern part of Africa. It is one of the oldest medicinal plants cited in Ayurveda promoting multiple health-beneficial effects like antidiabetic, anticancer, anti-inflammatory, antioxidant, antihyperlipidemic, antifungal, antibacterial (Yadav and Baquer, 2014; Arya *et al*., 2023), The fenugreek seed comprises fat (7%), protein (26%) and carbohydrates (58%) apart from dietary fibre, saponins and flavonoids (Gupta *et al*., 2021). The seed mucilage and gums consist of polysaccharides like galactomannans, applied in different pharmacological formulations (Kamble *et al*., 2013; Kumari *et al*., 2016; Iurian *et al*., 2017; Yasmeen and Shashikumar, 2019). Fenugreek seed was utilised for the treatment of palm oil mill effluent successfully as natural coagulants and flocculants respectively (Lanan *et al*., 2021). As biocoagulant aid, *T. foenum-graecum* contributed to the reduction in alum usage and yielded better performance in turbidity removal (Kashfi *et al*., 2018; Karnena and Saritha, 2022). Fenugreek extracts of leaves, seeds and stem displayed antimicrobial activity against *E. coli* and *Staphylococcus* (Premanath *et al*., 2011; Sharma *et al*., 2016 Hadi and Mariod, 2022). *T. foenum-graecum* showed inhibitory activity against multidrug-resistance *E. coli* and *S. aureus* bacterial strains with a MIC of 500 µg/mL and MBC of 1000 µg/mL. Antibiofilm activity of *T. foenum-graceum* methanolic extract was reported against *Pseudomonas aeruginosa* and *Aeromonas hydrophila* (Husain et al., 2015). *Moringa oleifera*, locally called ''drumstick' tree, belongs to Moringaceae family and is widely popular due to its nutritive and medicinal properties where all parts like the leaves, flowers, seeds, and pods have been utilized for centuries. It has healing properties to cure multiple diseases and microbiological infections (Desta and Bote, 2021). It has excellent biocoagulant properties in turbidity removal and in water purification (Islam *et al*., 2017; Aduro and Ebenso, 2019; Okuda and Ali, 2019). Significant coliform reduction of 89-96% and diminishing other potable characteristics features was also observed using *Moringa* extracts (Razis *et al*., 2014; Abiyu *et al*., 2018).

Hence, this study was ventured to investigate the applicability of natural seed based coagulants for water turbidity removal and bactericidal applications from household stored containers.

2. Experimental procedures

2.1. Sample collection

Trigonella and *Moringa oleifea* seeds along with Aluminum sulfate, [Al2(SO4)3·16H2O, alum were procured from the local market to compare performance of natural seeds with chemical coagulants. For analysing the performance of these biocoagulants, kaolin clay was also procured which mimics the surface water conditions.

2.2. Processing and De-fatting of the seeds

The seeds were washed with distilled water, sun dried and ground to fine powder using mixer grinder. The powder was sieved and stored in air tight containers for further use. The seed powder was defatted by treating with ethanol for 24 hrs and then centrifuging at 5000 rpm for 15 mins to separate out the seed cake from the supernatant. Fresh supernatant was collected each time before the start of the experiment to prevent any activity loss of the biocoagulant protein, if stored at 4 ^{0}C.

2.3. Preparation of synthetic water sample

4 gm of Kaolin powder was dissolved in 1 lit of distilled water to generate synthetic turbid water samples. The solution was stirred at medium speed for 1 hr to maintain uniformity in the clay dispersion with standing time of .24 hours for complete hydration of clay. The prepared solution was used to carry out turbidity removal studies.

2.4. Coagulation activity of seed by Jar Test

The conventional jar test apparatus was used to carry out the turbidity reduction test. Different doses of *Trigonella* seed and *Moringa oleifera* coagulants

(0.50 gm, 1.0 gm, 2.0 gm, 3.0 gm and 4.0 gm) were dissolved in prepared synthetic turbid water. Alum was added to one of the sample beakers, and for positive control no coagulant was added. After the coagulants were added to turbid water, the solutions were agitated at rapid mixing of 250 rpm for 20 minutes and slow mixing of 40 rpm for 15 minutes. After settling time of 30 mins, 3 ml of the respective samples from top 3 cm of water surface were withdrawn from each beaker and measured through ultraviolet-visible spectrophotometer at 500 nm. Initially kaolin clay turbidity was adjusted to 1.4-1.6 at 500 nm, optical density which corresponds to 450-500 NTU (high turbidity) at room temperature. Turbidity removal percentage was calculated as follows:

$$\% \text{ removal} = (A_0 - A_f)/ A_0 * 100$$

where, A_0 is initial absorbance and A_f is final absorbance after which there was no change in water clarity. Each experiment was performed in triplicates and the values are expressed as mean ± S.D.

2.5. Determination of water quality parameters

Parameters analysed were pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity, alkalinity and dissolved oxygen parameters. Turbidity was determined using a UV-Vis spectrophotometer at 500 nm while TSS was determined using the gravimetric method. The bacterial load and coliform count was estimated using Spread plate technique and Most Probable Number Method (MPN) using lactose broth to detect the presence of coliform (Bakare *et al*., 2003).

2.6. Coliform removal efficiency from surface water

In vitro antibacterial susceptibility of *Trigonella* seeds and *Moringa oleifera* were determined against bacterial strains isolated from the raw surface water. To assess bacteria removal, a beaker containing 1 L of synthetic turbid water (450-500 NTU/L) was spiked with *E. coli* (10^6 CFU/mL) without coagulant. Another beaker had optimal dosage of *Trigonella* seed and *Moringa oleifera* coagulants following the same coagulation procedure. The addition of bacteria had no effect on the turbidity. For bactericidal tests, a 200 μL sample was collected from below the water surface without disturbing the floc and spread on nutrient agar (for all bacterial specimens) and EMB plate (selective for *E. coli*). The plates were incubated for 24 hrs at 37 ^{0}C and results recorded.

Results and Discussion

Effect of biocoagulant dose on surface water turbidity

Different dosages of raw *Trigonella* seed powder (0.50 gm, 1.0 gm, 2.0 gm, 3.0 gm and 4.0 gm/lt) were initially chosen for optimization of turbidity reduction at pH 8.20 as shown in graph (4.3.1). Lowest dosages of 0.50 gm and 1.0 gm showed better performance than higher dosages because they increased the organic load in water making it more turbid. After defatting of *Trigonella* seeds by ethanol precipitation, the biocoagulant efficiency became comparable to that of alum. Alum gave turbidity reduction of 81.76 % and 95.29% after 1 hr and 24 hrs respectively whereas defatted biocoagulant exhibited 84.11% and 96.47% (for 0.5 gm), 68.23 % and 93.52% (for 1 gm) during the same contact time respectively. At the same dosage, non-defatted raw seed extracts reduced the performance. Similar results were obtained by Ramamurthy *et al*., 2012 and Kashfi *et al*., 2018 working on *Trigonella seed* and *A. gossypinus* which reflect that low doses of natural coagulants gave better performance in both high and low water turbidity. Ethanol precipitation did not hamper the efficiency of biocoagulant protein rather removal of organic components increased water clarity by 52.76%. Similar results were reported by Gidde *et al*. 2012; Amante *et al*. 2016, and Camacho *et al*., 2016, where they further confirmed that the residual seed cake after ethanol extraction retains the functionality of seed coagulant protein. Thus optimum dosage was determined to be 0.50 gm/l for *Trigonella* seed (Table 1). At dosage of 0.25gm, the *Moringa* seed coagulant had reduction of 72% and at dosage of 0.50 gm (Table 1); the turbidity reduction was 93%. Similar results were obtained by Bashir *et al*., 2016) where an optimum dosage of 50 mg/l of *Moringa oleifera* seed gave 88.7% of turbidity reduction. Gandiwa *et al*., 2020 reported a successful blend of natural coagulants with alum which resulted in reduced turbidity, pH, alkalinity and electrical conductivity within required limits set by WHO. According to WHO guidelines, turbidity less than 5 NTU for potable water is recommended which corresponds to O.D value less than 0.2 at 500 nm in spectrophotometer (Howard *et. al*, 2012; Yimer and Dame, 2021).

Table 1 Effect of seed dosage variations on water turbidity removal at pH 8.20

Coagulant Dosage	O.D (500m)	
	Trigonella	Moringa
0 gm	0.170	0.170
0.25gm	0.115	0.024
0.50gm	0.155	0.012
1.0gm	0.158	0.116
2.0gm	0.162	0.129
3.0gm	0.180	0.132
4.0gm	0.210	0.148
5.0gm	0.238	0.166

Effect of contact time of biocoagulants on water turbidity removal

Trigonella seeds yielded best performance with 96.47% reduction in surface water turbidity at contact time of 24 hrs using 0.25 gm seed. Comparable results were obtained with Alum, where 95.29 % turbidity reduction was observed. *Moringa* seeds yield 91.76% reduction after 24 hrs with 0.25 gm and 88.82% with 0.50 gm coagulant dose. 1 gm of *Trigonella* seeds also produced 92.90% turbidity reduction after 24 hrs, but the performance exhibited was less than 0.5 gm of the seed. Higher dosages did not yield better results than 0.5 gm and 1 gm due to addition of organic components generated from the biocoagulant itself. Defatted *Trigonella* seeds showed the best water turbidity removal activity at dosage of 0.25 gm after 24 hrs compared to *Moringa* (Fig. 2a, 2b). Kashfi *et al.*, 2018 reported using *T. foenum-graecum* (1 mg/L) as coagulant aid along with alum (15 mg/L) leading to turbidity removal of 58.9%. In the control water, slow turbidity changes were observed after 24 hrs. This observation highlights the existence of coagulating active compounds in the seed extract of *Moringa* and *T. foenum-graecum*, which maintains water clarity.

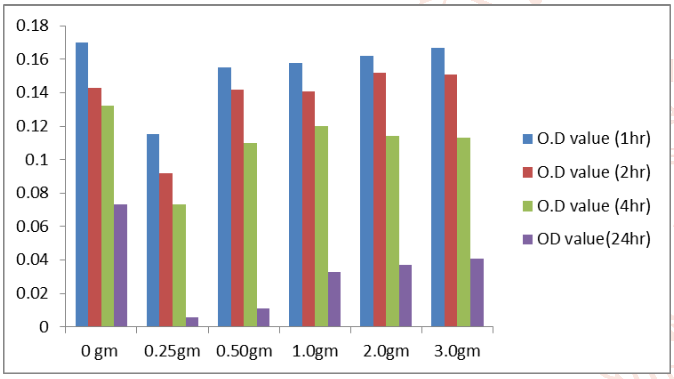

Fig. 2a) Effect of contact time on water turbidity removal of defatted Trigonella seed

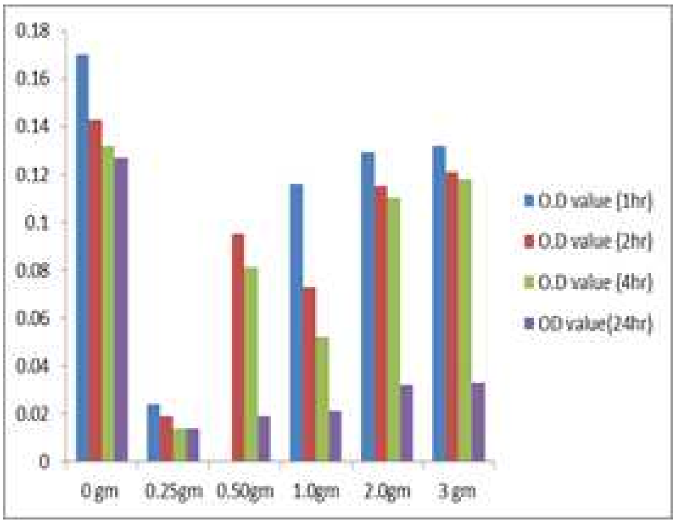

Fig. 2b) Effect of contact time on water turbidity removal of defatted Moringa seed

Effect of biocoagulants on pH change of water

pH is the determining factor for coagulation-flocculation processes. The pH of water sample without coagulant was 8.20. It was observed that after treatment with *Moringa* seed powder at 0.25 gm and 0.50 gm, pH of the solution decreased whereas pH increased at increasing dosage concentration. It can be concluded that basic amino acids of seed protein will accept proton from surface water and release hydroxyl group making the solution basic. *Moringa* gave lowest pH of 7.6 at 0.25 gm dosage. *T. foenum-graecum* seeds have the highest saponin and protein content whereas husk is high in polyphenols. Together they exhibit better antioxidant activity (Naidu et al., 2010). After addition of *T. foenum-graecum* seeds to water, pH reduced to 7.63 at dosage of 0.50 gm when OD value was measured at 500 nm (Fig. 3). Hence addition of the biocoagulants maintained drinking water pH in all cases and there was no abrupt pH change affecting human system.

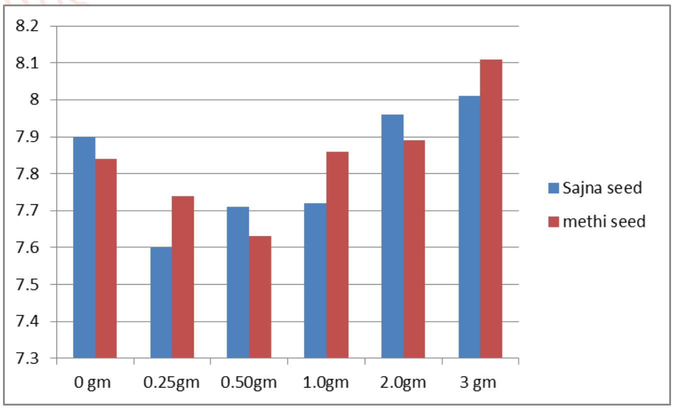

Fig. 3 Graph showing effect of seed biocoagulants on pH of water

Reduction of TS and EC in presence of biocoagulants

Total solids are the solid residues in a water sample after evaporation and weighing at 105 °C. They are used to calculate the amount of suspended solids (TSS) and dissolved solids (TDS) in water. TDS is the standard method for determining the concentration of dissolved ions whereas Electrical conductivity (EC) is a measure of conduction of electricity, through dissolved ions present in water bodies (Omer, 2020). The optimal range for TDS is between 500 and 1,000 mg/L, whereas EC should not be more than 1,500 µS /cm from a health perspective. The electrical conductivity of raw surface water altered (70-85% increase) with addition of Methi and *Moringa* seeds biocoagulants. There was detected increase in electrical conductivity values by dissociation of biocoagulants producing ions that raise electrical conductivity. *Moringa oleifera* dosage showed marked increase in conductivity as the dosage increased from 0 to 5 gm/L. The conductivity also increased in case of methi seeds as coagulant dosage

increased from 0 to 5 gm/L. when compared to chemical coagulants like alum, natural biocoagulants have lower dissociation capacity in water. In case of *Moringa* seeds, at pH ~8, the proteins dissociate to produce ions in solution and lead to increase the conductivity value. Protein exists in zwitter ion state and acts as polyelectrolyte compound with amine and carboxylic group. Normally when protein dissociate in water, it releases positive ion (H+) which leads to increased conductivity. For potable water maximum permissible limits for electrical conductivity is within the range 1000 μS/cm or 1 mS/cm and our results are within suitable range of permissible limits.

Reduction of DO and BOD in presence of biocoagulants

Biochemical oxygen demand (BOD) is an indication of high bacteria pollution in water, which breaks down and devours complex organic matter to simpler compounds utilizing oxygen for cellular processes. DO indicate dissolved oxygen parameter, a high value of which signifies good health of water body. Taiwa *et al* 2020, reported reduction in BOD value from 6.61 to 1.68 mg/L (74.6% reduction) after treatment with 80 mg/L of *Moringa* protein. Shan *et al*., 2017 reported 50% reduction of BOD after the usage of *Moringa* seed cake. Simultaneously the DO improved from 2.58 ± 0.01 to 4.00 ± 0.00 mg/L. Our report shows 25.55% reduction in BOD value using *Moringa* and 18.37% reduction using *Trigonella* respectively. The values comply with drinking water quality standards of 2 mg/L or less (WHO, 2008). Water with BOD values of 10 mg/L indicates water pollution whereas 3–5 mg/L is considered moderately clean.

Bacterial load and Coliform reduction in presence of biocoagulants

Biological pollution also causes water to be unsuitable for human consumption and can spread illnesses like diarrhoea. As water is often kept in containers in rural homes, treating it with these natural biocoagulants is required to lower the bacterial burden. Earlier literature report shows that usage of *Moringa* seed powder has considerable reduction in counts of *E. coli*, Yeast, *Pseudomonas* and Coliforms (Karnena *et al*. 2021, 2022). Sapana *et al*., 2012 also confirmed antimicrobial nature of *Moringa oleifera* seed, reducing coliform count from 400,000 to 100 CFU/ml after treating ground water. At 40 mg/L of *Moringa* protein, bacterial growth inhibition was distinct against *Klebsiella edwardsii, Pseudomonas aeruginosa, Klebsiella ozoenae, Alcaligenes feacalis* and *Klebsiella pneumonia* (Taiwo et al., 2020). Similar antibacterial results of *Moringa* coagulant proteins were reported by Ghebremichael *et al*., 2005; and Ferreira *et al*., 2011. Moyo *et al*., 2012 with MIC value close to 6.25 mg/mL Al-Timimi, 2019 reported highest activity of *Trigonella* seed extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zones as 22 mm and 17 mm respectively. Similar antibacterial and antifungal findings of *Trigonella* seed oil were reported by Hadi and Mariod, 2022 *T. foenum-graecum* exhibited good antimicrobial activity against *S. aureus* MRSA, *Bacillus subtilis* and *Candida parapsilosis* at lower concentrations (Alwhibi and Soliman, 2014; Kumari *et al*., 2016). Radini *et al*., 2018 displayed antibacterial effects of *Trigonella* seeds iron nanoparticles. At 40 mg/L Moringa protein, bacterial growth inhibition was distinct against *Klebsiella edwardsii, Pseudomonas aeruginosa, Klebsiella ozoenae, Alcaligenes feacalis* and *Klebsiella pneumonia* (Taiwo *et al*., 2020). Our antibacterial test results utilizing *Trigonella* and *Moringa* seeds revealed 96% and 84% reduction in microbial load after 2 hrs treatment of the tested water sample plated in nutrient agar plate (Fig. 4).

Fig. 4 Nutrient agar plate showing effect of Moringa and Trigonella seeds on reducing bacterial load from surface water

Performance of *Moringa and Trigonella* seeds on reducing bacterial load

Coliforms are bacterial microbes present in the environment and in the faeces of all warm-blooded animals and humans. Most pathogenic bacterial microbes that contaminate water supplies come from the fences of humans or

animals. Testing drinking water is necessary to make it coliform free and restore its potability. Reduction in the total coliform count was evident from the EMB agar plate picture (Fig. 5). The main reason could be removal in turbidity in eliminating pathogens from drinking water. Microbes remain attached to larger suspended particles and settling with flocs helps to remove them also. Total coliform counts were determined after treatment with respective biocoagulants from the clarified water. Very significant removal of total coliforms was found after treatment with natural seed based coagulants. The reduction of total coliform counts was about 61.48%, and 62.22 respectively using *Moringa oleifera* and *Trigonella foenum-graecmu* respectively (Fig. 5). Similar results were reported by Choubey *et al*., 2012; where using *M. oleifera,* 96 % total coliform reduction in synthetic raw water was seen. The waters treated with *M. oleifera* showed a good reduction. Indeed, according to some studies, the protein of *M. oleifera* seeds has a very interesting antibacterial activity (Santos et al., 2013; Nhut et al., 2021). The activity of this peptide against pathogens, including *Pseudomonas* and *Streptococcus*, has been well studied by Suarez et al. (2005). Simultaneously turbidity removal from 100 NTU to 5.9 NTU was also observed.

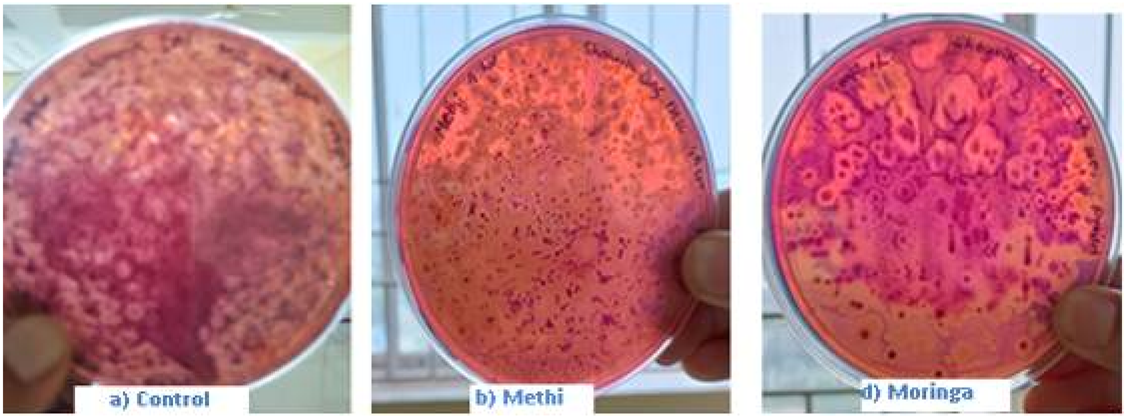

Fig. 5 EMB agar plate showing effect of biocoagulants on reducing Coliform load from surface water

Discussion and Conclusion

Trigonella foenum graecum and *Moringa oleifera* markedly reduced the turbidity of the raw water but compared to alum the turbidity removal efficiency was less. Alum made the water acidic which is undesirable among other negative impacts. Usage of seed biocoagulants balanced and maintained the pH of drinking water effective at an optimum dosage of 0.25-0.50 gm/L. Coliform removal was effective and was maintained for more than 24 hrs. These seeds offer a sustainable cost effective approach to the rural people for obtaining potable water in small-scale POU (Point Of Use) at their household. Scale up processes or commercialization can also be sought by blending seed coagulants with alum and using it as coagulant aid. Thus there is an immediate need for thorough and in-depth studies to analyse the mechanism of action of natural coagulants and develop strategies to deploy them for large-scale water treatment.

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